

AMENDMENT

Listing of Claims

This Listing of Claims is intended to replace all prior listings and versions of claims in the application. Since this is a reissue application and all pending claims are shown as compared to the issued patent, for the convenience of the Office, a marked-up copy of the claims showing amendments being made relative to versions pending prior to entry of this Amendment is attached hereto as Appendix A.

1. An isolated polynucleotide encoding a FEN-1 polypeptide as shown in SEQ ID NO:1 or SEQ ID NO:3, or a fragment of said polypeptide having flap endonucleolytic cleavage activity.

2. An isolated polynucleotide, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:29-51.

3. An isolated polynucleotide of **claim 2**, wherein said polynucleotide comprises the sequence of SEQ ID NO:28.

4. A host cell comprising the polynucleotide of **claim 1**.

5. A non-mammalian host cell comprising a mammalian FEN-1 polypeptide of **claim 1**.

6. The polynucleotide of **claim 1** that is full length.

21. A method of detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising the steps of:

(a) contacting, under conditions in which a FEN-1 polypeptide exhibits cleavage activity, a sample suspected of containing a target nucleic acid comprising the predetermined target sequence with:

(i) a 5'-polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region that is capable of specifically hybridizing under said cleavage conditions to a first portion of the predetermined target sequence and a 5'-region located immediately 5' to the 3'-region; and

(ii) a 3'-polynucleotide probe comprising a 5'-region that is capable of specifically hybridizing under said cleavage conditions to a second portion of the predetermined

target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,

such that the 3'-region of the 5'-probe and the 5'-region of the 3'-probe specifically hybridize immediately contiguously with one another to the first and second portions, respectively, of the predetermined target sequence to form a 5',3'-double flap structure cleavable by a FEN-1 polypeptide;

(b) cleaving the 5',3'-double flap structure with a FEN-1 polypeptide; and

(c) detecting the presence or absence of, and/or quantifying the amount of, FEN-1 polypeptide-generated cleavage, thereby detecting the presence of the predetermined target sequence in the sample.

22. The method of **claim 21** in which the 5'-probe contains a detectable label.

23. The method of **claim 22** in which the 5'-region of the 5'-probe contains the detectable label.

24. The method of **claim 23** in which the 5'-end of the 5'-probe contains the detectable label.

25. The method of **claim 21** in which the 5'-probe is immobilized on a support.

26. The method of **claim 21** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

27. The method of **claim 26** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO:28.

28. The method of **claim 21** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

29. The method of **claim 21** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

30. The method of **claim 21** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

31. The method of **claim 21** in which the 3'-region of the 3'-probe is 1 to 10 nucleotides in length.

32. The method of **claim 21** in which the 3'-region of the 3'-probe is 1 nucleotide in length.

33. The method of **claim 21** in which the 5'-region of the 5'-probe is 1 to 5 nucleotides in length.

34. The method of any one of **claim 21-33** in which the amount of FEN-1 polypeptide-generated cleavage is quantified.

35. The method of any one of **claims 21-33** in which the presence or absence of FEN-1 polypeptide-generated cleavage is detected.

51. A substrate cleavable by a FEN-1 polypeptide comprising:

(a) a bridge polynucleotide comprising a first portion and a second portion located immediately 3' to the first portion;

(b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region and a 5'-region located immediately 5' to the 3'-region; and

(c) a second polynucleotide probe comprising a 5'-region and a 3'-region located immediately 3' to the 5'-region,

wherein the 3'-region of the first probe and the 5'-region of the second probe are specifically hybridized immediately contiguously with one another to the first and second portions, respectively, of the same bridge polynucleotide molecule, thereby forming a substrate cleavable by a FEN-1 polypeptide.

52. The substrate of **claim 51** in which the first probe contains a detectable label.

53. The substrate of **claim 52** in which the 5'-region of the first probe contains the detectable label.

54. The substrate of **claim 53** in which the 5'-end of the first probe contains the detectable label.

55. The substrate of **claim 51** in which the first probe is immobilized on a substrate.
56. The substrate of **claim 51** in which the 3'-region of the second probe is 1 to 10 nucleotides in length.
57. The substrate of **claim 56** in which the 3'-region of the second probe is 1 nucleotide in length.
58. The substrate of **claim 51** in which the 5'-region of the first probe is 1 to 5 nucleotides in length.
59. A kit for use in detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising:
- (a) a FEN-1 polypeptide;
 - (b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a first portion of the predetermined target sequence and a 5'-region located immediately 5' to the 3'-region; and
 - (c) a second polynucleotide probe comprising a 5'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a second portion of the predetermined target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,
- wherein the 3'-region of the first probe and the 5'-region of the second probe are capable of specifically hybridizing immediately contiguously with one another to the first and second portions, respectively, of the predetermined target sequence to form a 5',3'-double flap structure that is capable of being cleaved by the FEN-1 polypeptide.
60. The kit of **claim 59** in which the first or second probe contains a detectable label.
61. The kit of **claim 59** in which the FEN-1 polypeptide contains a detectable label.
62. The kit of **claim 59** in which the 3'-region of the second probe is 1 to 10 nucleotides in length.
63. The kit of **claim 59** in which the 3'-region of the second probe is 1 nucleotide in length.

64. The kit of **claim 59** in which the 5'-region of the first probe is 1 to 5 nucleotides in length.

65. The kit of **claim 59** in which the first probe contains a detectable label.

66. The kit of **claim 65** in which the 5'-region of the first probe contains a the detectable label.

67. The kit of **claim 66** in which the 5'-end of the first probe contains the detectable label.

68. The kit of **claim 59** in which the first or second probe is immobilized on a substrate.

69. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

70. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO. 28.

71. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

72. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

73. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

74. The method **claim 21** in which the 5'-region of the 5'-polynucleotide probe is 20 nucleotides in length.

75. The substrate of **claim 51** in which the 5'-region of the first polynucleotide probe is 20 nucleotides in length.

76. The kit of **claim 59** in which the 5'-region of the first polynucleotide probe is 20 nucleotides in length.

77. A method of detecting the presence of a predetermined nucleotide sequence in a sample, comprising:

contacting a sample suspected of containing a polynucleotide comprising the predetermined nucleotide sequence with (i) oligodeoxyribonucleotide probes capable of forming a 3',5'-double flap cleavage substrate in the presence of the predetermined nucleotide sequence and (ii) means for cleaving the 3',5'-double flap cleavage substrate, wherein the contacting is performed under conditions in which the oligodeoxyribonucleotide probes anneal with the predetermined nucleotide sequence, if present in the sample, to yield the 3',5'-double flap cleavage substrate; and

detecting cleavage of the 3',5'-double flap cleavage substrate, thereby detecting the presence of the predetermined nucleotide sequence in the sample.

78. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

79. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide encoded by a polynucleotide comprising SEQ ID NO. 28.

80. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide comprising the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

81. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide comprising the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

82. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide comprising the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

83. A method of detecting the presence of a predetermined nucleotide sequence in a sample, comprising:

contacting a sample suspected of containing a polynucleotide comprising the predetermined nucleotide sequence with (i) oligodeoxyribonucleotide probes capable of forming a 3',5'-double flap cleavage substrate in the presence of the predetermined nucleotide sequence and (ii) a FEN-polypeptide encoded by a polynucleotide according to any one of **claims 1-3**, wherein the contacting is performed under conditions in which the oligodeoxyribonucleotide probes anneal with the predetermined nucleotide sequence, if present in the sample, to yield the 3',5'-double flap cleavage substrate; and

detecting cleavage of the 3',5'-double flap cleavage substrate, thereby detecting the presence of the predetermined nucleotide sequence in the sample.

84. A method of detecting the presence of a predetermined sequence in a sample, comprising:

contacting a sample suspected of containing a polynucleotide comprising a predetermined sequence with first and second oligodeoxyribonucleotide probes and a FEN-1 polypeptide, wherein: (i) the first oligodeoxyribonucleotide probe comprises a 5'-flap region and a 3'-region complementary to a first region of the predetermined sequence; (ii) the second oligodeoxyribonucleotide probe comprises a 3'-flap region and a 5'-region complementary to a second region of the predetermined sequence that is located downstream of, and contiguous to, the first region of the predetermined sequence; (iii) the FEN-1 polypeptide comprises a sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and fragments thereof having 5'-flap endonucleolytic cleavage activity; and (iv) the contacting is performed under conditions in which the first and second oligodeoxyribonucleotide probes anneal with the predetermined sequence, if present in the sample, to form a 3',5'-double flap substrate cleavable by the FEN-1 polypeptide; and

detecting cleavage of the 3',5'-double flap substrate, thereby detecting the presence of the predetermined sequence in the sample.